Invesitgation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Reports of in-house investigations of **Sodium Propionate** (missing), **Glutamic Acid Hydrochloride & Zinc Gluconate** (missing) in the developing chicken embryos 3/23/78

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Hearing Clerk, HFC-20

DATE: MAR 23 1978

L.F.M.

FROM : Leo F. Mansor
GRAS Review Branch, HFF-335

SUBJECT: Reports of Chicken Embryo Investigations

Attached are the reports of in-house investigations in the developing chicken embryo on the following substances:

1. Sodium propionate
2. Glutamic acid hydrochloride
3. Zinc gluconate

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

DATE: March 1, 1978

THRU: HFF-150

FROM : Supervisory Chemist
Whole Animal Toxicity Branch (HFF-155)

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigation of glutamic acid hydrochloride in the developing chicken embryo.

M. Jacqueline Verrett

M. Jacqueline Verrett, Ph.D.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Glutamic Acid Hydrochloride

Protocol:

Glutamic Acid Hydrochloride (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

lutamic acid hydrochloride showed moderate toxicity when administered via the air cell at 96 hours. The calculated LD_{50} was 19.0 mg/kg (0.950 mg/egg). With the other three conditions of test no LD_{50}s could be calculated.

Scattered abnormalities were observed under all conditions of test, but in no instance were serious abnormalities significantly higher than or different from those observed in the background. Glutamic acid hydrochloride displayed no teratogenicity under the test conditions employed.

1. L-Glutamic Acid Hydrochloride, Stauffer Chemical Co., Westport, Ct.

2. McLaughlin, J., Marliac, J.P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770

3. Verrett, M. J., Marliac, J.P., and McLaughlin, J., Jr., (1964) JOAC 47, 1002-1006

4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

GLUTAMIC ACID HYDROCHLORIDE
AIR CELL AT 0 HOURS

TABLE 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
20.00	400.00	120	52.50	5.00	0.00
10.00	200.00	120	36.66	3.33	0.83
5.00	100.00	120	35.00	2.50	0.00
2.50	50.00	120	29.16	1.66	0.00
1.250	25.00	120	47.50	8.33*	3.33
Water		115	39.13	1.73	0.00
Control		341	9.38	0.87	0.58

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$

GLUTAMIC ACID HYDROCHLORIDE
AIR CELL AT 96 HOURS

TABLE 2

Dose mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	30	100.00*	0.00	0.00
5.00	100.00	30	100.00*	0.00	0.00
2.50	50.00	110	87.27*	2.72	0.00
1.250	25.00	110	70.00*	2.72	0.90
0.6250	12.50	110	54.54*	1.81	1.81
0.250	5.00	105	24.76	1.90	0.95
0.1250	2.50	105	16.19	2.85	2.85
Water		120	26.66	3.33	2.50
Control		341	9.38	0.87	0.58

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 18.9977 mg/kg (0.950 mg/egg)

GLUTAMIC ACID HYDROCHLORIDE
YOLK AT 0 HOURS

TABLE 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
20.00	400.00	117	65.81	0.85	0.85
10.00	200.00	120	74.16	3.33	1.66
5.00	100.00	119	84.03*	2.52	2.52
2.50	50.00	118	67.79	0.84	0.84
1.250	25.00	120	68.33	0.00	0.00
Water		115	65.21	0.86	0.00
Control		341	9.38	0.87	0.58

*Significantly different from solvent $p \leq 0.05$
**Slope not significantly different from zero $p = 0.05$

GLUTAMIC ACID HYDROCHLORIDE
YOLK AT 96 HOURS

TABLE 4

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	105	40.95	4.76	3.80
5.00	100.00	105	40.95	4.76	1.90
2.50	50.00	105	40.95	2.85	0.95
1.250	25.00	105	42.85	3.80	1.90
0.6250	12.50	105	50.47	4.76	1.90
Water		120	40.00	0.83	0.83
Control		341	9.38	0.87	0.58

**Slope is negative